REMARKS

By this amendments claims 1-8 and 10 are revised, claim 9 is canceled, and new claims 15-21 are added to place this application in immediate condition for allowance. Currently, claims 1-8, 10, and 15-21 are before the Examiner for consideration on their merits. Claims 11-14 stand withdrawn from consideration.

First, claim 9 is incorporated into claim 1.

Second, new claim 15 is added and support for this claim is found in the specification on page 4, lines 24-27.

Third, the claims are revised in response to the Examiner's request for the proper use of "A" and "The".

Fourth, the claims are revised to remove the language associated with "preferentially" and new claims 16-21 are added to cover the embodiments removed from the claims in this regard.

Lastly, Applicants traverse the rejections in light of the revision to claim 1 and the traverses are set out below under the headings of Anticipation and Obviousness.

ANTICIPATION

The Examiner asserts that each of XP00203193 (Derwent), WO 96/32929 (WO), and United States Patent No. 5,102,783 to Alkemade et al. (Alkemade) anticipates claim 9 under 35 U.S.C. § 102(b).

While the Examiner may have been able to question which elements are required in claims 1 and 9, the revision to claim 1 now makes it clear that each of the recited components is a required part of the preservation medium. The change to claim 1 raises the question of whether any of the applied prior art establishes a *prima facie* case of anticipation.

Applicants assert that the rejection based on 35 U.S.C. § 102(b) is no longer appropriate for claim 1.

Derwent discloses a preservation medium that includes a hyaluronic acid that is derived from microorganisms so that it does not meet the limitation that it is free of a component of animal origin. This alone means that it cannot anticipate claim 1.

Secondly, Derwent does not teach a medium that contains amino acids and vitamins. This is another reason why Derwent cannot anticipate claim 1 as now amended and the rejection based on this reference must be withdrawn.

WO relates to a preservation medium that is similar as possible to natural body liquid for in vivo application. WO is directly opposite the invention because the invention relates to a medium for the preservation of living cells in an in vitro application. The environments of in vivo and in vitro are different from each other, sharing no common composition or property. This is emphasized by the limitations that the medium of claim 1 now includes amino acids and vitamins. These components are not found in the medium of WO and a *prima facie* case of anticipation cannot be said to exist for this reason.

The same argument made for WO applies to Alkemade. Alkemade is also a medium for in vivo application and the medium of Alkemade cannot be said to anticipate claim 1.

Based on the above, the rejection of claim 1, as amended, based on Derwent, WO, and Alkemade must be withdrawn.

OBVIOUSNESS

Since the applied prior art cannot anticipate claim 1, the question remains as to whether any of the prior art can be used to formulate a rejection based on 35 U.S.C. § 103(a) and obviousness.

Derwent cannot be modified to meet the limitations of claim 1 and cannot form the basis for an obviousness allegation. Derwent can only be used at +4 °C and has to be changed every 24 hours. This medium cannot be used routinely for the preservation of corneas or entire ocular globes.

The medium of the invention is more complex than that of Derwent and can be used for preservation, organ culture, cellular culture, freezing, transport, and deturgescence of living organs, biological tissues, and cells, and in particular living human corneas. The preservation medium according to the invention is usable at temperatures ranging between -196 °C and 37 °C in particular.

In this regard, attached herewith is an article that describes the effect of the preservation medium of the present invention. Using the inventive preservation medium, corneas can be preserved for 30 days in organoculture (31-37 °C). The

preservation can attain 7 days for functional CD34+ at 4 ºC under hypoxis and hypercania. For the transport of total blood at ambient temperature, the duration can attain 8 days. The preservation medium can also be used in cryogeny.

The stability can be attained by the combination of all of the elements of claim 1 and without the use of components derived from animal origin as is found in the prior art. The use of the combination of components of trace elements, amino acids, vitamins, and a stabilizing pH buffer, in addition to the high molecular weight hyaluronic acid and a sodium chloride ensures the metabolic preservation of the organs, biological tissues, etc.

Since the prior art cannot anticipate claim 1 in its amended form, the question of obviousness relates to whether any of the prior art could be modified to include the claimed components so as to produce the invention. Again, since Derwent says nothing about the claimed components, the reasoning for modifying Derwent from Derwent's own teachings does exist and Derwent cannot be used to formulate a rejection based on 35 U.S.C. § 103(a).

WO and Alkemade also fail to provide any reasoning to arrive at the invention through modification of Derwent. The technical field of Alkemade relates to a substitute for the serums used when culturing or freezing living tissues. In Alkemade, hyaluronic acid is used as a substitute for the serums typically used in the prior art. This has nothing to do with the invention or Derwent. Thus, Alkemade provides no reason to modify Derwent and arrive at the invention.

Moreover, since Alkemade is merely concerned with using an alternative to serums when culturing or freezing living tissues, there is no reason to include the components of claim 1 therein and Alkemade cannot form the basis for a rejection based on 35 U.S.C. § 103(a).

WO relates to a solution useful for minimizing the trauma of surgery. This is unrelated to Derwent or Alkemade and WO cannot provide any basis to modify either of these references so as to arrive at the invention. Moreover, since WO is not similar to the invention in terms of its use, there is no reason to modify the medium of WO and arrive at the invention.

The teachings of Alkemade and WO do not give any inspiration in either composition or function of the medium of the present invention. Due to differences between the environments of in vivo and in vitro, a person skilled in the art is unable to predict that a hyaluronic acid as a natural component of the aqueous humor could protect cells in vitro and thus is unable to predict the use thereof in a medium. Therefore, the technical solution of the present invention cannot be gleaned from Derwent, WO, and Alkemade and an allegation of obviousness based on this prior art cannot be made.

In light of the above arguments, it is submitted that the prior art does not establish a *prima facie* case of anticipation and cannot be used to allege obviousness.

Accordingly, the Examiner is requested to examine this application and pass all claims under consideration onto issuance.

Further, since claims 11-14 include the limitations of claim 1 therein and claim 1 is patentable over the prior art, the restriction requirement should be withdrawn and these claims passed onto issuance with claims 1-8 and 10.

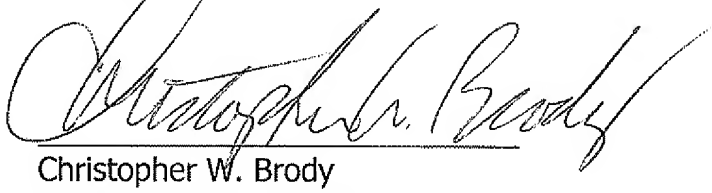
If the Examiner believes that an interview would expedite the allowance of this application, the Examiner is invited to telephone the undersigned at the number below.

The above is considered to be a complete response to all issues raised in the outstanding Office Action.

Again, reconsideration and allowance of this application are requested.

Applicants petition for a three month extension of time. Please charge Deposit Account No. 50-1088 the fee of $555.00. Please charge any fee deficiency or credit any overpayment to Deposit Account No. 50-1088.

Respectfully submitted,
CLARK & BRODY



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Date: January 4, 2010

Attachments to Amendment filed 01/04/2010
U.S. Application No. 10/562,902
Applicant: Daniel Licari et al.
Docket No. 71247-0051

2 pages attached

MAINTENANCE OF THE FUNCTIONAL CD34+ CELLS AT 4°C UNDER HYPOXIA AND HYPERCAPNIA

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OBJECTIVES: Improvement of the conservation of the hematopoetic progenitors and stem cells (CD34+ cells) in hypothermia (+4°C) in the liquid medium by incubation in gas mixture comprising low O2 (hypoxia) and increased CO2 (hypercapnia) concentrations.

MATERIALS: CD34+ cells isolated from CB were incubated in conservation medium Stem-α S3 (Stem alpha, Saint Genis l'Argentiere, France) for 10 days at +4°C under hypoxia (5% O2) and hypercapnia (9% CO2) or air (20% O2 and 0,05% CO2). Conservation medium was used without cytokine or with hIL3.

RESULTS: Incubation at hypoxia and hypercapnia resulted in 60% (64±10%, n=19) conservation of the CD34+ cells (with respect to starting cell number) comparing to only 30% (37±8%, n=19) preserved at air. This condition also assure the significantly better preservation of the committed hematopoietic progenitors (34±9% comparing to 18±6% at air) detected by CFC assay. These effects were maintained in the presence of hIL3 in the conservation medium.

This advantage obtained at hypoxia and hypercapnia enabled three fold higher expansion capacity per harvested CD34+ cell in the secondary culture.

More efficient conservation at 5% O2 and 9% CO2 in the terms of viable cell number and the individual functional capacities of CD34+ cells, was associated with less apoptose (AnnexinV/PI assay) and significantly better preservation of cells in G1/G0 phase (Ki67/PI staining). The higher frequencies of primitive aldehyde dehydrogenase (ALDH) expressing cells were detected after conservation at hypoxia and hypercapnia comparing to air. Scid Repopupalating Cells (SRCs) were maintained in this condition.

CONCLUSIONS: Combine effects of hypoxia and hypercapnia assure better maintenance of functional hematopoietic progenitors and stem cells at +4°C in the liquid medium. These results suggest a way to optimise cell conservation without freezing for the use in cell therapy and regenerating medicine.

CONSERVATION DES PROGENITEURS HEMATOPOIETIQUES 24H APRES DECONGELATION D'UN GREFFON GRACE AU MILIEU STEM ALPHA.S1

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Objectif de l'étude : Au laboratoire, il est précieux de pouvoir conserver à court terme un échantillon décongelé d'un greffon pour faire une analyse complémentaire ou confirmer des résultats. Actuellement, les conditions de conservation des échantillons de sangs de cordon (SC) ou de Cellules Souches Périphériques (CSP) décongelés sur 24h ne sont pas réunies. Les expériences ont montré que la viabilité des CD34+ analysée par Cytométrie en Flux est souvent correcte alors que la fonctionnalité des progéniteurs hématopoiétiques révélée par la culture cellulaire est souvent diminuée voire inexistante. Nous avons donc étudié l'intérêt à utiliser un milieu sans composant d'origine animale ni humaine, le milieu Stem Alpha. S1, pour la conservation des échantillons.

Matériel et Méthodes : 3 SC et 3 CSP (1 LNH et 2 Myélomes) ont été étudiés. L'échantillon est décongelé au bain marie à 37°C puis dilué au 1/10ème par addition d'une solution (Sol D) comprenant 40% de NaCl 0.9%, 40% d'Albumine Humaine à 4% et 20% d'ACD-A. Cet échantillon est immédiatement analysé (J0). Le reste des cellules est conservé 24h à 8°C, soit dans la Sol D, soit en milieu Stem Alpha.S1 sans rouge de phénol (Société Stem Alpha, France) et est réanalysé. La viabilité cellulaire est mesurée au bleu trypan. L'analyse des CD34 est effectuée selon la procédure définie par l'ISHAGE en double plateforme, à J0 et J1 pour les sangs de cordon conservés dans la Sol D, à J0 pour les CSP. La technique de cultures cellulaires est réalisée en milieu Stem alpha.4B, gel semi solide à base de collagène humain. La suspension cellulaire mise en culture est définie pour cultiver 200 CD34+viables/ml (pour les CSP, le chiffre retenu est celui obtenu lors de la décongélation). Le milieu de culture est réparti dans une boite 4 puits NUNC. Après 14 jours en incubateur à 37°C en atmosphère humide et CO_2, les gels sont récupérés sur lame de verre, séchés et colorés au May-Grünwald-Giemsa. La lecture des CFU-GM et BFU-E est réalisée sous microscope optique x10.

Résultats : Résultats exprimés en médiane et valeurs minimum et maximum par greffon

		Viabilité		Leucocytes viables x10^6		CD34 x10^6
		Sol D	SAlpha S1	Sol D	SAlpha S1	Sol D
SC n=3	J0	73,5 (67-80)		25,85 (22,21-29,76)		5,85 (3,15-10,36)
	J1	58 (55-60)	**96 (85-97)**	3,16 (1,19-4,56)	**15,47** (1,44-15,95)	5,9 (2,68-7,4)
	Rdt	*78,9*		*12,22*	*59,85*	*100,9*
CSP n=3	J0	92 (81-93)		196,24 (8,6-351,46)		
	J1	34 (18-37)	**96 (78-99)**	21,26 (1,73-59,4)	**46,73** (8,34-57,82)	
	Rdt	*36,96*		*10,83*	*23,81*	
		CFU-GMx10^4		BFU-Ex10^4		
		Sol D	SAlpha S1	Sol D	SAlpha S1	
SC n=3	J0	18,68 5,69-31,66		59,975 (7,32-112,63)		
	J1	9,38 (0-10,55)	**24,8 (0-49,6)**	10,03 (0,22-14,68)	**52,94** (0,32-65,82)	
	Rdt	*50,23*	**>100**	*16,72*	*88,27*	
CSP n=3	J0	636 (2113-207)		1859 (152-2113)		
	J1	0 (0-508)	**283 (221-530)**	0 (0-59,4)	**366** (135-984)	
	Rdt		*44,5*		*19,68*	

Conclusions : nous observons un vrai bénéfice à employer le milieu Stem Alpha. S1, pour conserver sur 24 h les progéniteurs hématopoiétiques des SC et CSP décongelées. Cette méthode de conservation pourrait permettre le transport des échantillons et leur analyse différée à l'AFSSAPS.